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condenser is greater in the present arrangement than it would be in the other with no ground wire, that on the last condenser is much less. Hence the present arrangement is the better of the two. The advantage of having the voltage on  $C_1$  almost twice the peak voltage of the transformer is preserved here, because we preserve the feature that is essential for this result, namely, that one end of the transformer is connected to the middle point of the first D. C. condenser line. The resonance condensers, described in the previous article, for eliminating errors due to small changes of the generator speed, can be used as they were there. In cases where the steadiest voltage is not needed and the voltage is not too high, we may cut off the filament current from one kenotron and run the tube between the other side of the line and the ground. This is useful if we need to ground one end of the tube.

For experimental tests of this apparatus I have inserted a current-type voltmeter in each of the ground lines from the middle points of the condensers, and also have used telephones in these lines. The voltmeters not sensitive enough to show the current except in  $C_1$ , and the telephones are not good for quantitative work expecially in the presence of stray 500 cycle noises, but the currents and, therefore, the voltage fluctuations are apparently of the right order of magnitude. A point illustrated by these telephone experiments is that if the voltage is raised until brush discharges occur on the line, they produce irregular fluctuations of voltage that can be clearly heard in the telephone. Anyone building an outfit of this type should take all precautions needed to avoid brush discharges and should listen for them with a telephone before relying on the steadiness of the potential.

<sup>1</sup> Webster, D. L., these Proceedings, 6, 1920, 26-35.

THE EFFECT OF THE CONCENTRATION OF NITRATES ON THE REDUCING POWERS OF BACTERIA¹

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During the course of some investigations on bacteria pathogenic to insects, I frequently used the nitrate-nitrite reduction test as one of a large number of differential characters. Sometimes the tests for nitrites were positive; at other times negative with the same species of microörganism. This led me to believe that the concentration of the salt in the medium was important; in other words, that very definite amounts of salt were necessary in order that the physiological activity of reduction might manifest itself.

As shown in the accompanying table a number of species of micro-

organisms were selected. Witte's peptone media, containing various molecular concentrations of NaNO<sub>3</sub> and KNO<sub>3</sub>, were prepared. These media were inoculated with equal amounts of 48-hour cultures of the bac-

Table Showing Results of the Nitrate-Nitrite Reduction Tests
Code: A plus sign indicates reduction of nitrates to nitrites
A cipher indicates the lack of such reduction

WG indicates a weak growth. NG indicates no growth

SPECIES OF ORGANISM		.0002 M	.0005 м	.0008 м	.001 M	.01 M	.1 M	.5 м	1 M	2 M	4 M
Spirillum metchnikovi	NaNO <sub>3</sub> KNO <sub>3</sub>	0	+++++++++++++++++++++++++++++++++++++++	++	++	+++	++	W.G. 0 0 W.G.	0	N.G. 0 0 N.G.	0
Bacillus prodigiosus	NaNO <sub>3</sub> KNO <sub>3</sub>	0	0 0	++	++	+++	++	++++	+++	N.G. 0 0 N.G.	N.G. 0 0 N.G.
Bacillus coli communis	NaNO <sub>3</sub>	0	0 0	0 0	0 0	+++	++	W.G. + +	W.G. 0 0 W.G.	0	0
Coccobacillus acridi- orum. Variety "Souche Sidit"	NaNO <sub>3</sub> KNO <sub>3</sub>	0	0 0	0 0	0	+++	++	+++	++	W.G. 0 0 W.G.	N.G. 0 0 N.G.
Coccobacillus acridi- orum. Variety "Souche Cham"	NaNO <sub>3</sub>	0	0	0	0	++	+	++	W.G. 0 0 W.G.	<b>0</b> 0	0
Bacillus anthracis	NaNO <sub>3</sub> KNO <sub>3</sub>	0 0	0 0	0	0	0 0	0	0 0 W.G.	W.G. 0 0 W.G.	0 0	0
Staphylococcus pyogenes albus	NaNO <sub>3</sub> KNO <sub>3</sub>	0 0	0 0	0	0 0	0 0	0 0	0	0 0	W.G. 0 0 W.G.	0
Streptococcus disparis	NaNO <sub>3</sub> KNO <sub>3</sub>	W.G. 0 0 W.G.	0	W.G. 0 0 W.G.	0	0 0	0	W.G. 0 0 W.G.	N.G. 0 0 W.G.	N.G. 0 0 N.G.	N.G 0 0
Checks (No bacteria)	NaNO <sub>3</sub> KNO <sub>3</sub>	0 0	$\frac{0}{0}$	0 0	0 0	$\begin{vmatrix} \frac{1}{0} \\ 0 \\ 0 \end{vmatrix}$	0 0	0 0	$\begin{vmatrix} \frac{1}{0} \\ 0 \\ 0 \end{vmatrix}$	0 0	0 0

teria concerned and incubated for five days. After this time the cultures were tested for the presence of nitrites by the use of the sulphuric acid, potassium iodide, starch reaction and the naphthylamine, sulphanilic acid, acetic acid test. Of course, the absence of nitrites may not indicate non-reduction of nitrates, since the nitrites formed prior to the test may have been reduced to free nitrogen or ammonia. Nevertheless, in that case a nitrate reaction should not be obtained. The presence or absence of nitrates after five days growth was tested in each case by evaporating the culture to dryness and by adding phenolsulphonic acid, diluting with water and transferring to a Nessler jar. Sodium hydroxide solution was added to make it alkaline and the color reactions compared.

The table shows that each species capable of reduction has a distinct range. Bacillus prodigiosus has a greater range than Spirillum Metchnikovi, but the latter has the power of reducing nitrates in a weaker concentration. Prodigiosus, on the other hand, has the power of reduction in greater concentrations of the salts. This inability of Metchnikovi to reduce in greater concentrations may be associated with the inhibiting influence of high osmotic pressure on growth, for it will be seen that Metchnikovi grew weakly in concentrations greater than 0.1 mol. That growth and ability to reduce are not always associated, however, can be seen in the case of B. coli communis which grew poorly in 0.5 mol. concentration and reduced NaNO<sub>3</sub>.

A distinct range of reduction power in various concentrations can also be noted for some of the other species. Some have a smaller, some a greater range. In so far as the reducing power is concerned no difference was noted between sodium and potassium ions. Nevertheless, a slight difference between sodium and potassium ions in the amount of growth was observed. In 4 mol. NaNO<sub>3</sub> Coccobacillus acridiorum variety "Souche Cham" grew poorly, but no growth whatever was obtained in the same concentration of KNO<sub>3</sub>. The same condition was observed when the Anthrax bacillus was used. This difference in ions was further observed by using B. coli communis, where the sodium salt gave a poor growth at 0.5 mol. concentration and the potassium salt a good growth at the same concentration. The difference between ions and growth is not dependent upon variations in the number of bacteria originally introduced into the cultures for the amounts were previously carefully calibrated.

<sup>1</sup> Contribution from the U. S. Bureau of Entomology in coöperation with the Bussey Institution of Harvard University. (Bussey Institution, No. 173.)